

THE BIOLOGY OF FERTILIZATION AND BROOD PROTECTION IN SPIRORBIS (LAEOSPIRA) MORCHI¹

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The described species of the serpulid genus *Spirorbis* are hermaphroditic and exhibit brood protection. Alexander Agassiz (1866) appears to be the first investigator to offer information on the biology of fertilization in the genus. Agassiz, who examined *Spirorbis spirillum*, claimed that the embryos reached quite an advanced stage within the body of the parent before the brood sac was deposited within the cavity of the tube. Fewkes (1885) examined what he thought to be *Spirorbis borealis* and observed that the eggs were laid in strings within the parental tube; however, he was not able to confirm Agassiz' claim that development initially occurs within the body of the parent. According to Schively (1897), who worked with what she believed to be *Spirorbis borealis*, the eggs pass into the body cavity and then into the operculum where fertilization takes place and a capsule is secreted. Schively further claimed that once the egg capsule was secreted, the capsule passed through an opening in the operculum and was placed in the "mid-dorsal furrow." It seems quite likely that both Fewkes and Schively were working with *Spirorbis spirillum* and not *Spirorbis borealis*. Zur Loye (1908) believed that fertilization in *Spirorbis borealis* occurred externally within the parental tube. Abe (1943), working with a species which he identified as *Spirorbis argutus*, reported that fertilization probably took place internally. Recently, Gee and Williams (1965) have reported that fertilization in *Spirorbis borealis* and *Spirorbis pagenstecheri* occurs externally and have presented evidence that self-fertilization is possible in both species.

Brood protection in the genus *Spirorbis* takes place either within the parental tube or within a modified operculum. As far as is known, the mode of brood protection is species-specific. Thorson (1946) has claimed that *Spirorbis granulatus*, which is characterized as an operculum brooder (Caullery and Mesnil, 1897; Bergan, 1953b), broods in the tube and suggested that the mode of brood protection varies within the species; however, Thorson (personal communication) believes that he may have been mistaken as to the identity of the species. Of the two types of brood protection, opercular brood protection is the most specialized and has been considered a recent development in the evolution of the genus (Elsler, 1907; Borg, 1917; Gravier, 1923). Bergan (1953a) observed the presence of a pore in the operculum of *Spirorbis granulatus*, but was of the opinion that it was too small to allow the passage of eggs and did not pursue its significance. To date, there has been no attempt to explain how spawned oocytes are transferred to the opercular brood pouch.

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The present paper deals with the biology of fertilization and opercular brood protection in *Spirorbis* (*Lacospira*) *morchii* Levinsen. The observations on fertilization are an elaboration of those presented earlier in abstract (Potswald, 1964).

MATERIALS AND METHODS

Spirorbis morchi adults were collected periodically throughout the year, from 1960 to 1963, in Argyle Creek on San Juan Island, Washington. The animals were found on rock and shell and often in association with *Spirorbis* (*Paraderiospira*) *vitreus*. In Argyle Creek, *Sp. morchi* breeds the year around; consequently, developmental stages were always available.

In order to determine whether animals raised in strict isolation are capable of self-reproduction, individual larvae were isolated and cultured. This was accomplished by placing larvae in polyethylene ice-cube trays, one larva per cube, and allowing them to settle. Each cube was given a number, thereby allowing complete records to be kept for each isolate. The sea water used in these cultures was filtered to prevent larvae, that might be in the sea water system, from settling in the containers used to store the water, and was then allowed to stand for a minimum of three weeks before using. Such water was considered to be sperm-free and will be subsequently referred to as sperm-free sea water. The sea water was removed periodically, by means of a pipette attached to an aspirator, and replaced. A new pipette was used for each cube, thus eliminating the possibility of cross-contamination by introducing sea water from one cube into that of another. Initially, food was supplied in the form of *Nitzschia* sp.; however, the cultures fouled very rapidly even upon the addition of small numbers of diatoms. It was found that the animals maintained themselves on protozoa and bacteria present in the water so the addition of diatoms was discontinued.

Larvae used in the isolation experiments were artificially released from brood pouches. Opercular brood pouches, which contained actively moving larvae, were selected and removed with #5 watchmaker's forceps. Once removed, the opercular ampullae were torn open with forceps or size 0 insect pins. The larvae thus released showed the same behavioral responses and settled in about the same length of time as naturally released larvae.

The histological techniques used in the present study were reported previously (Potswald, 1967). In addition, the chloranilic acid method of Carr *et al.* (1961) was used for the demonstration of calcium.

OBSERVATIONS ON FERTILIZATION

Attempts at controlled fertilization

In the summer of 1961, while in residence at the Friday Harbor Laboratories, several experiments were carried out in an attempt to bring about controlled artificial fertilization. Adults with gravid female segments were removed from their tubes, individually isolated in syracuse dishes, and gently washed with two or three changes of pasteurized sea water to remove any extraneous sperm that may be adhering to the body. Male and female segments were then jabbed a few times with a size 0 insect pin which caused the longitudinal muscles of the abdomen to contract strongly and spasmodically, resulting in the release of gametes through

ruptures in the body wall. After a minute or two, the oocytes were removed by means of a pipette and transferred through several changes of pasteurized sea water to prevent possible polyspermy. Periodic examination with the compound microscope revealed that in over half the animals tested, activation had been initiated, as manifested by the lifting of a fertilization envelope; however, in none of the cases observed was there germinal vesicle breakdown accompanied by polar body formation. The same results were obtained when sperm from another individual were added. In all cases, the sperm were active and the oocytes, as judged by morphological criteria, appeared to be "ripe." It is possible that physiological maturation of the oocytes precedes natural spawning by only a matter of hours as has been shown by Howie (1961) for *Arenicola marina*.

Isolation of larvae

A second approach involved the use of artificially released larvae which were individually isolated and raised in sperm-free sea water (see Materials and Methods). Seventy larvae were isolated in the spring and summer of 1961. The mor-

TABLE I
Brooding record for Spirorbis morchi adults raised from larvae in isolation

Animal	Date isolated as larva	Date found brooding	Age at first brooding	No. of embryos	Remarks
I-12	6/18 61	9 06 62	15 mos.	3	Non-viable brood
I- 6	4/07 61	9/20 62	17 mos.	4	Non-viable brood
I-15	7/21 61	12/ 8 62	17 mos.	5	Non-viable brood
		2/22 63		3	Larval release & settlement
III- 4	7 03 61	9 06 62	14 mos.	4	Larval release & settlement
		10/22 62		5	Larval release & settlement
		12/13 62		?	Operculum & brood shed before completion of development
		3 09 63		?	Non-viable brood

ality rate was quite high during the first year and as of August, 1963, there were only 16 survivors. Of these, only four produced broods and as can be seen from the data summarized in Table I, two of the four isolates produced more than one brood.

Among the four animals which were able successfully to mature and spawn gametes, only a small number of eggs per brood were produced. This small number, and the fact that only $\frac{1}{4}$ of the survivors were able to spawn at all, can probably be attributed to nutritional factors. As mentioned earlier, attempts to supply food, in the form of diatoms, were not successful, and therefore discontinued. Obviously, settled animals can be maintained on bacteria and protozoa present in the sea water; however, only a few will be able to mature a small number of gametes under these conditions. At the time the cultures were discontinued (March, 1964), 12 of the original isolates still survived and ranged from 31 to 33 months in age. One of the isolates was sectioned and although it had the adult number of segments, all abdominal segments were completely agametic.

The cause of non-viable brood formation is not known. In the above cases, it is quite possible that mature sperm were not formed and, consequently, the oocytes not fertilized. It should be pointed out, however, that non-viable broods are often found in animals taken from the field. The fact that viable larvae, capable of normal settlement and metamorphosis, were produced from animals isolated as larvae is conclusive proof that in *Sp. morchi*, reproduction in isolation is possible. Had more effort and time been spent in finding a proper food source, it might be assumed that all of the isolates would have produced viable broods, probably over quite an expanse of time.

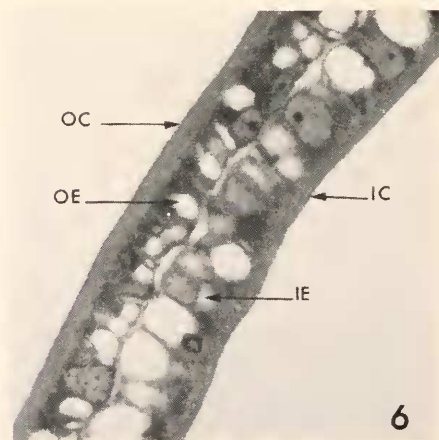
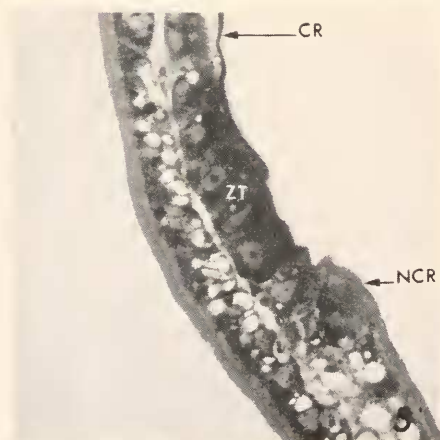
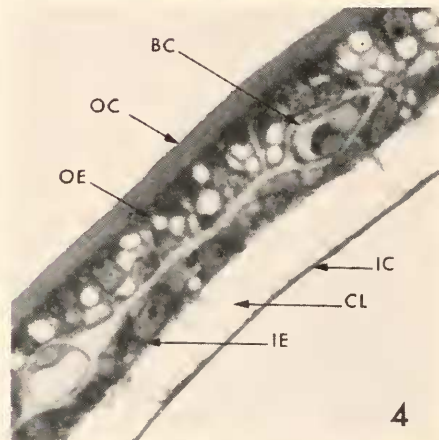
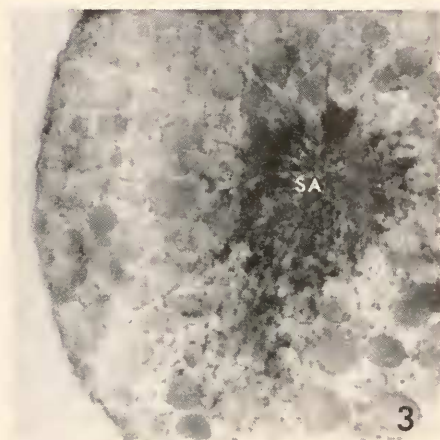
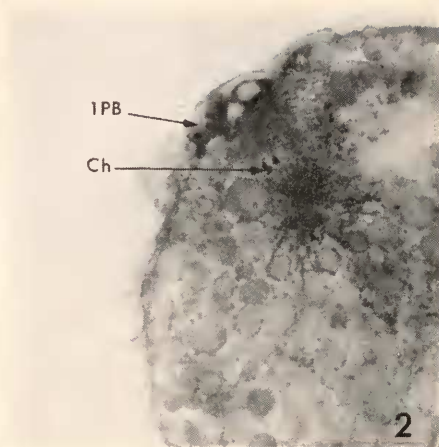
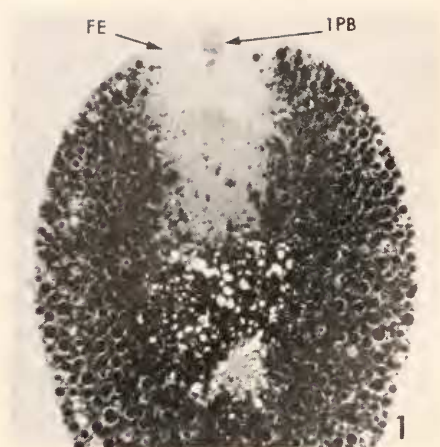
Isolation of adults

To test sperm viability, contents of male segments were artificially released, placed in filtered sea water, kept at about 12° C., and periodically examined with the compound microscope. Mature sperm remain active during the first eight

TABLE II
Isolation of Spirorbis morchi adults

Animal	Date of isolation	Date of spawning	Time elapsed since isolation in days	Remarks
1	6/18/62	7/05/62	17	Arrested development
2	6/18/62	6/23/62	5	Larvae released 7/24/62
*3	6/18/62	6/22/62	4	Larvae released 7/24/62
4	6/19/62	6/22/62	3	Arrested development
5	6/19/62	7/09/62	20	Arrested development
6	6/22/62	6/25/62	3	Arrested development
7	6/26/62	7/01/62	5	Larvae released 7/26/62
8	6/29/62	7/01/62	2	Larvae released 8/01/62
9	6/29/62	7/04/62	5	Arrested development
10	7/05/62	7/13/62	8	Larvae released 7/12/62
*11	7/12/62	7/26/62	14	Arrested development
12	2/07/63	2/14/63	7	Arrested development
13	2/07/63	2/17/63	10	Arrested development
*14	2/07/63	2/17/63	10	Larvae released 3/25/63
15	2/07/63	3/13/63	34	Arrested development
		4/06/63	58	Fixed 4/06/63
16	2/07/63	2/19/63	12	Larvae released 3/23/63
		3/27/63	48	Larvae released 4/25/63
17	2/07/63	3/12/63	33	Arrested development
18	2/07/63	2/13/63	6	Arrested development
		3/20/63	41	Larvae released 4/19/63
19	2/07/63	2/17/63	10	Arrested development
20	2/07/63	2/12/63	5	Larvae released 3/20/63
		3/27/63	48	Arrested development
21	2/07/63	3/20/63	13	Arrested development
22	2/07/63	3/09/63	30	Larvae released 4/13/63
23	2/07/63	3/06/63	27	Arrested development
*24	8/12/63	8/14/63	2	Larvae released 9/24/63
*25	8/12/63	9/05/63	25	Larvae released 10/17/63
		11/7/63	84	Non-viable
26	8/19/63	8/22/63	3	Arrested development

* Animal left in tube.



FIGURES 1-6.

hours after release, but become completely non-motile within 24 hours. "Plates" of spermatids remain active for three days but start to disintegrate on the fourth day without completing metamorphosis. With these facts in mind, adult *Sp. morchi*, taken from the field and in most cases removed from their tubes, were isolated in either 10-ml. beakers or polyethylene ice-cube trays containing pasteurized sea water. Daily observations were made to determine the length of time elapsed after isolation for spawning to occur. Of 63 isolates, 26 spawned, and of these, 13 produced broods of normal larvae. The results are summarized in Table II.

It will be subsequently explained that *Sp. morchi* adults removed from their tubes are not always capable of transferring all of their spawned oocytes into the opercular ampulla, and therefore, a number, and often all, of the oocytes are spawned freely into the sea water. Development outside of the brood pouch is almost always suspended, presumably because of the growth of bacteria and protozoa which adhere to the sticky fertilization envelope. In most instances, the term "arrested" where it appears in the above table refers to such cases.

In the adult isolation experiments, newly spawned oocytes were removed intermittently from culture and examined with the compound microscope. Sperm penetration was never observed and only a partial sequence of events can be given. In the earliest stage observed, the vitelline envelope was just elevating at the animal pole as the fertilization envelope. The germinal vesicle at this time appears to be still intact and lies close to the surface under the plasma membrane of the animal pole. About $2\frac{1}{2}$ hours after elevation of the fertilization envelope, extrusion of the first polar body begins and is completed in about 30 minutes. The second polar body is given off about two hours after the first and with its extrusion, the first polar body divides. In live material, fine filaments can be seen extending from the surface of the polar bodies to the fertilization envelope where they apparently attach; the filaments have not been seen in fixed material. Because of the large amount of heavily pigmented yolk, male and female pronuclei have not been observed following polar body extrusion. The fertilization envelope remains closely

FIGURE 1. Section through a fertilized oocyte showing the lifting of the fertilization envelope (FE), and first polar body (1 PB). Epon; Richardson's stain. (300 \times)

FIGURE 2. Section through an oocyte spawned after 58 days in isolation, showing the first polar body (1 PB) and two chromosomes (Ch) arranged in second meiotic metaphase. Paraffin; haematoxylin-eosin. (750 \times)

FIGURE 3. An adjacent section to that in Figure 2 showing a sperm aster (SA). Paraffin; haematoxylin-eosin. (750 \times)

FIGURE 4. Cross-section through the calcified region of the operculum showing the outer cuticle (OC), outer epithelium (OE), inner epithelium (IE), calcified layer (CL), inner cuticle (IC), and blood capillary (BC). Epon; Richardson's stain. (1125 \times)

FIGURE 5. Cross-section through the operculum in the zone of transition (ZT) between the calcified region (CR), and the non-calcified region (NCR). Epon; Richardson's stain. (750 \times)

FIGURE 6. Cross-section through the non-calcified region of the operculum showing the outer cuticle (OC), outer epithelium (OE), inner epithelium (IE), and inner cuticle (IC). Epon; Richardson's stain. (1125 \times)

applied to the egg surface except at the animal pole where the perivitelline space is about 14 microns (Fig. 1). The above observations were made at a temperature of 12–13° C.

Five oocytes spawned after the parent had been in isolation for 58 days (animal #15, second spawning) were fixed and sectioned. Cytological examination revealed that the first polar body had been given off and the chromosomes were in second meiotic metaphase; a sperm aster was present in the cytoplasm (Figs. 2, 3). Although actual fusion of pronuclei has not been observed, it is, nevertheless, concluded that *Sp. morchi* is not only capable of reproduction in isolation but of self-fertilization. The fact that both polar bodies are given off in oocytes spawned in isolation supports this conclusion and makes it seem unlikely that parthenogenesis is occurring.

OBSERVATIONS ON OPERCULAR BROOD PROTECTION

Structure of the operculum in Sp. morchi

Second of the branchial tentacles in position, in relation to the dorsal midline, the operculum arises from the left branchial lobe (see zur Loye, 1908, Fig. 12). Typically, the operculum contains a spacious cavity, the ampulla, and is capped by a characteristically bilobed calcareous plate. Careful observation of a living operculum with a dissecting microscope reveals the presence of a distinct though small pore in the wall of the ampulla. It can be determined with direct lighting that, except for the ampullar side, the operculum is almost completely calcified. At the base of the operculum is a short stalk, the peduncle, which often has a groove running around its circumference. Below the groove, when present, the peduncle is swollen. This swelling is the replacement operculum and will become a functional operculum after the extant operculum is shed. The size of the operculum in mature adults can be quite variable but averages about 700×330 microns at its longest and widest dimensions. No attempt has been made to correlate opercular size with, for example, age classes, but it is possible that such a correlation may exist.

From the outside in, the operculum consists of an outer cuticle and epithelial layer followed by an inner epithelial layer and cuticle. There is a distinct space separating the two epithelial layers; however, a basement lamina has not been demonstrated. The histology of the calcified region of the operculum is somewhat different from that of the non-calcified portion.

In the calcified region (Fig. 4), the outer cuticle is about 2–2.2 microns thick and has a striated appearance. The outer surface of the cuticle is fuzzy, suggesting the presence of minute projections. The cuticle is strongly PAS-positive, indicating the presence of a polysaccharide, and stains blue with Heidenhain's Azan. The outer epithelial layer varies in height from 3 to 6 microns and is cuboidal. Protoplasmic processes extend from the apices of the cuboidal cells into the outer cuticle and give the latter its striated appearance. Nuclei are basal in position, and contain a single nucleolus and irregular pieces of chromatin. Several vacuoles are located within each cell of the outer layer but their significance is not known. Separating the outer epithelial layer from the inner epithelial layer is a space of about 3 microns which widens to about 6–7 microns at the sites of blood capillaries which penetrate between the two tissue layers. The inner epithelial layer is about

half as thick as the outer and contains few vacuoles. Nuclei of the inner layer are more elliptical in shape than those of the outer layer. The inner cuticle is about 1 micron thick and very dense; separating it from the inner epithelium is a space which varies from 6 microns along the sides of the operculum to about 28 microns at its apex. Coarse protoplasmic processes extend from the inner epithelium across the space and attach to the cuticle. Sections of opercular brood pouches fixed in

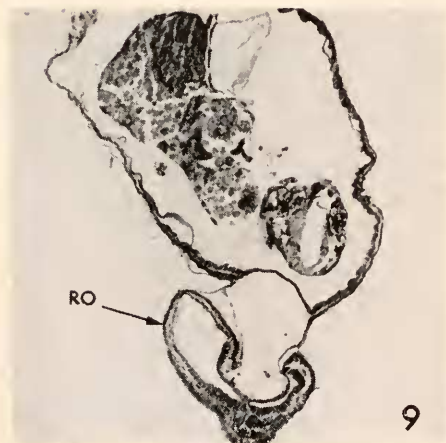
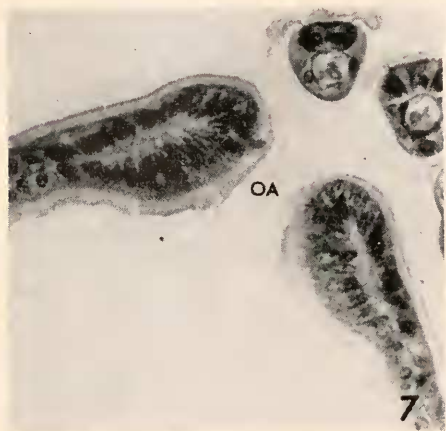


FIGURE 7. Cross-section through the opercular aperture (OA). Note that the epithelium surrounding the aperture is columnar. Paraffin; iron haematoxylin. (400 \times)

FIGURE 8. Sagittal section through an operculum containing a brood. Note the opercular aperture (OA), and opercular peduncle (OP). Paraffin; iron haematoxylin. (75 \times)

FIGURE 9. Sagittal section through an operculum containing a moribund brood. Note the developing replacement operculum (RO). Paraffin; haematoxylin-eosin. (75 \times)

FIGURE 10. Cross-section through the branchial crown showing that the opercular aperture opens to the center of the crown. Paraffin; haematoxylin-eosin. (75 \times)

neutral formalin and treated with the chloranilic acid method reveal that the space between the inner epithelium and cuticle is the site of calcification. The surface of the inner epithelial layer, facing the cuticle, gives a very strong reaction and presumably it is this layer that is responsible for calcium secretion.

There is a sharp zone of transition between the calcified and non-calcified regions of the operculum (Fig. 5). In the non-calcified portion, the outer cuticle and epithelium are similar to those of the calcified region (Fig. 6). The space separating the outer epithelium from the inner epithelium averages 1–3 microns and there are fewer blood capillaries than are found in the calcified region. The inner epithelium varies from about 7 to 9 microns in thickness and is also cuboidal in appearance. Nuclei are tall, having a height almost equivalent to that of the entire cell. One or more large intracellular vacuoles are present, but often disappear when the operculum contains a brood. In both epithelial layers of the calcified and uncalcified regions alike, the cells contain within their cytoplasm several dark-staining spherical bodies, each of which encloses a less dense internum. The dark-staining bodies are removed in the solvents used in routine paraffin embedding which suggests that they are lipid in composition. The inner cuticle of the uncalcified region is like the outer except the surface is less fuzzy and there is a thin membrane-like layer applied to it.

Around the opercular aperture the epithelial layers become considerably thickened (Fig. 7). The cuboidal nature of both outer and inner epithelium changes to columnar. The height of the outer cells averages about 13 microns and that of the inner about 22 microns. Nuclei are elongated in shape and the cytoplasm is almost completely devoid of vacuoles. The interspace between outer and inner epithelial layers is obliterated. Muscle and nerve fibers are not demonstrable.

The outer cuticle and epithelium of the operculum are continuous with those of the peduncle (Fig. 8). The inner cuticle and epithelium forms the floor of the operculum and is not continuous with any portion of the peduncle; consequently, the only opening into the opercular brood pouch is by way of the opercular pore or aperture. There is a basement lamina below the columnar epithelium of the peduncle and also below the inner epithelium of the operculum where the latter forms the roof of the peduncle. Composing the core of the peduncle are longitudinal muscle fibers and blood capillaries embedded in an ill-defined connective tissue containing fibers which stain blue with Azan. According to Hanson (1949) in the peduncle and branchial tentacles of *Sp. corrugatus* and *Sp. militaris* three nerves can be demonstrated using the Azan method; however, in the present material, using the same technique, only one nerve (the internal branchial nerve, using Hanson's terminology) could be demonstrated. At the top of the peduncle, or base of the operculum, muscle fibers can be observed to penetrate between the inner and outer epithelial layers of the operculum, but they can not be followed for any distance. As mentioned earlier, there is often a groove running around the circumference of the peduncle. This may properly be referred to as the zone of detachment because it is here that the operculum becomes disconnected from the peduncle when it is periodically shed. A new or replacement operculum makes its appearance below the old operculum and is generally well-formed before the latter is shed (Fig. 9). Experimental extirpation of the operculum results in its renewal in the same position.

Brooding behavior in Sp. morchi

The primary or first operculum formed at metamorphosis of the larva does not serve as a brood pouch but is replaced by a secondary operculum which does serve in that capacity. This conclusion is based on observations made on animals raised from isolated larvae. It cannot be stated exactly how many consecutive broods an operculum can accommodate before it is replaced, but it is certainly at least three. As pointed out by Gravier (1923), opercular replacement is not peculiar to operculum brooders but also occurs periodically in species which brood in the tube. Based on counts made on 115 broods, the average number of embryos per brood in *Sp. morchi* is 15; however, within a population, a range of from 3 to 45 embryos per brood is not unusual. The embryos are not surrounded by a common membrane within the operculum as described in *Sp. granulatus* by Bergan (1953a).

On three different occasions larval release was observed in the laboratory. Larvae emerge *via* the opercular pore which opens wide enough to allow the simultaneous release of two larvae at a time. After release of the larvae, the pore appeared to close somewhat but it could not be ascertained whether or not this closure was complete. In all three cases, within 12 hours of larval release a new brood had been deposited in the operculum and the opercular pore was tightly closed. From these observations, it is not possible to determine whether the pore opens actively during larval release or merely passively as the result of force applied by

TABLE III

Number of eggs spawned free and/or successfully transferred to the brood pouch in Spirorbis morchi adults removed from their tubes

Animal	Numbers of eggs spawned	Numbers of eggs placed in brood pouch	Numbers of eggs spawned free into the sea water
1	8	0	8
2	12	3	9
3	37	18	19
4	13	2	11
5	21	0	21
6	5	5	0
7	16	7	9
8	15	0	15
9	10	0	10
	11	0	11
10	5	0	5
	8	0	8
11	4	0	4
12	7	0	7
	8	6	2
13	13	0	13
14	13	0	13
	3	0	3
15	8	0	8
16	6	6	0
17	7	4	3
18	9	1	8

the moving larvae; however, it is certain that the pore closes after receiving a brood and this would seem to suggest an active process. It should be pointed out that in a virgin operculum the pore is tightly closed prior to deposition of the first brood and this would suggest that opening, like closing, of the pore is under active control.

Earlier it was mentioned that adults removed from their tubes are inefficient in transferring spawned eggs into the opercular brood pouch. In most instances, counts of the number of eggs spawned free and the number placed in the opercular brood pouch were made. These data are tabulated in Table III.

In all cases, the oocytes spawned free into the sea water were activated and commenced development. Failure to transfer spawned oocytes into the brood pouch has never been observed in control animals left in their tubes.

Although egg transfer has never been observed, a reasonable hypothesis can be constructed based upon the aforementioned observations. First, it has been established that the only entrance into the brood chamber is *via* the opercular pore; consequently, the oocytes must be spawned to the outside and then transferred into the operculum. The opercular pore, in some unexplained way, is capable of opening and closing. Activation of the oocyte apparently occurs outside of the operculum. An animal removed from its tube is inefficient in transferring its eggs into the operculum; consequently, the confines of the tube are necessary. It seems reasonable to suppose that gametes are released *via* rupture of the body wall (Potswald, 1967) into the space formed by the ventral fecal pellet groove and wall of the tube. The oocytes are here fertilized and are swept, by means of ciliary action, across the achaetous zone, forward along the dorsal surface of the thorax, and finally into the center of the branchial crown which is completely withdrawn into the tube at spawning. Having reached the branchial crown, the fertilized eggs pass into the operculum by means of the opercular pore which opens to the center of the crown (Fig. 10).

Inefficiency in egg transfer in the case of adults removed from their tubes is not total. As can be seen from an examination of the data, a number of animals were successful in transferring a portion of their brood and, in fact, two were 100% effective in transferring relatively small broods. *Spirorbis morchi* has a well developed thoracic cloak which extends posteriorly and attaches ventrolaterally on about the sixth abdominal segment. The under surface of the cloak is ciliated and could conceivably simulate, to some degree, the confines of the missing tube. The latter explanation would hold true especially for animals which assume an abnormal position, *i.e.*, ventral surface of the thorax facing the substratum, in which case the cloak would serve as a natural trough into which the spawned oocytes could fall. It is common for animals removed from their tubes to assume such a position.

A major problem yet to be solved involves the controlling factors operating in opening and closing of the opercular pore. Perhaps control is endocrine in nature and correlated with spawning. At the level of light microscopy, histological investigation failed to reveal muscle fibers in the area of the pore. In an attempt to determine the possible cytological basis of contraction, a study of the opercular epithelium, at the ultrastructural level, is presently being undertaken.

DISCUSSION

Functional hermaphroditism is found in nearly all phyla of invertebrates (Coe, 1943). Generally, when eggs and sperm develop concurrently in the same animal there are mechanisms involved which insure that self-fertilization does not take place. Nevertheless, there are documented cases of self-fertilization in various invertebrate groups. Smith (1950) has demonstrated, quite conclusively, self-fertilization in *Neanthes lighti*, an hermaphroditic, viviparous nereid which occurs along the California coast, from estuaries and sloughs of higher than normal salinity to rivers where salinity may be less than 2% that of sea water. Within the phylum Mollusca there are many instances where self-fertilization has been demonstrated, especially among the pulmonate gastropods where individuals may reproduce by self-fertilization generation after generation when experimentally isolated (see Coe, 1943, 1944 for review). Nyholm (1951) has shown that in *Labidoplax buskii* (Echinodermata, Holothuroidea) artificial self-fertilization can occur on a limited scale during the height of the breeding season. Finally, in the Arthropoda there is evidence that self-fertilization is possible in some barnacles (Barnes and Crisp, 1956; Barnes and Barnes, 1958).

In the examples cited it is obvious that eggs and sperm from the same individual are physiologically compatible; however, in most of the cases mentioned, cross-fertilization is probably the normal mode of reproduction, or at least it occurs much of the time. Whenever selfing is occasional or periodic, it need not cause any essential change in the evolutionary pattern of the species; the gene pool continues to be a reality. If, however, self-fertilization becomes obligatory rather than facultative, pure lines or clones are produced which do not exchange genes; the genotype of each line becomes a closed system capable of changing only through mutation or through reversal to biparental sexuality (Dobzhansky, 1951). In the latter case, the species, by virtue of selfing, robs itself of much evolutionary plasticity and actually gives up the benefits of sexual reproduction.

The observations presented in this report on the biology of fertilization in *Sp. morchi* are, to some extent, comparable to the observations of Gee and Williams (1965) on *Sp. borealis* and *Sp. pagenstecheri*. Although Gee and Williams did not rear animals from settlement to maturity in isolation, they did obtain data from isolated adults indicating that *Sp. borealis* and *Sp. pagenstecheri* are capable of self-fertilization. Gee and Williams make the assumption that cross-fertilization occurs when a number of worms are placed together and point out that in both species the viability of the progeny produced in association is noticeably greater in comparison with the progeny produced in isolation. This decrease in viability may be due to semi-lethal recessive genes. An apparent decrease in viability is also observed when *Sp. morchi* is allowed to reproduce in isolation.

Dasgupta and Austin (1960) report that they have evidence which suggests that the hermaphrodite serpulids *Spirorbis* and *Filograna* have been derived from triploids with a loss of a single chromosome. According to their study, *Serpula crater* has a diploid number of 14, and considering the "ancestral serpulid" as being $2n = 14$ then *Spirorbis* and *Filograna* are $2n + n - 1 = 20$. Dales (1963), presumably on the basis of the latter work, has made the interesting suggestion

that self-fertilization may have arisen because of the difficulty of chromosome pairing. Studies such as Dasgupta and Austin's which suggest the evolution of a group through polyploidy are often based, as is theirs, on chromosome numbers alone without study of chromosome morphology, and, as White (1954) points out, hermaphroditic forms capable of self-fertilization do not seem to show any more polyploidy than those in which cross-fertilization is obligatory. Although, at present, there is no concrete evidence that *Spirorbis* normally cross-fertilizes, there are cogent arguments in favor of this view. One of the best arguments, aside from the genetic implications discussed above, is that offered by Gee and Williams (1965) who argue that the gregarious settling behavior exhibited by *Spirorbis* larvae (Knight-Jones, 1951) would seem to allow cross-fertilization in a population. Conceivably, an animal, just prior to spawning, could draw into its tube, by means of the respiratory current, sperm from an adjacent animal which has already spawned. It is concluded that *Spirorbis* is capable of self-fertilization and this may be of some advantage to a sedentary hermaphroditic species which has a non-planktotrophic dispersal phase; however, cross-fertilization is probably the common mode of reproduction in a natural population.

Elsler (1907) described opercular brood protection in *Sp. corrugatus* Montagu and *Sp. pusillus* de Saint Joseph as taking place between the calcareous plate and the opercular epithelium. Borg (1917) found that brood protection in *Sp. pagenstecheri* occurs in the same fashion as described by Elsler. Apparently, the structure of the operculum in the last-mentioned species must be quite different from that in *Sp. morchi* and is deserving of further study. Bergan (1953a) describes brood protection in *Sp. granulatus* as occurring in the opercular ampulla as it does in *Sp. morchi*. Bergan also observed the presence of the opercular pore; however, he was of the opinion that it was too small to allow the passage of eggs and did not attempt to determine its function. In the present study on *Sp. morchi*, it has been observed that the opercular pore is capable of opening and closing, and, in fact, is the only entrance into the opercular ampulla. The mechanisms by which the pore operates have yet to be elucidated.

Elsler (1907), Borg (1917), and Gravier (1923) all speculated that opercular brood protection is a recent development as compared with brood protection in the tube. According to Borg, brood protection in the operculum has been found only in species having three thoracic segments and never in species having $3\frac{1}{2}$ or four thoracic segments. This would fall in line with the idea proposed by Caullery and Mesnil (1897) that the genus *Spirorbis* has been evolved from other Serpulidae by a gradual incorporation of thoracic segments into the achaetous region. Although a very interesting hypothesis, and one worthy of consideration, it would be of interest to know what advantage, if any, opercular brood protection has over brood protection in the tube. Perhaps a comparative physiological study of embryos developing under the two different modes of brood protection might shed some light on this problem.

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SUMMARY

1. Data obtained from *Spirorbis morchi* larvae reared from settlement to maturity in isolation, together with observations on isolated adults, provide evidence that *Sp. morchi* is capable of self-fertilization. Self-fertilization in *Spirorbis* is believed to be facultative and not obligatory.

2. The histology of the opercular brood pouch in *Sp. morchi* is described, and it is concluded that the only opening into the brood pouch is by means of an opercular pore.

3. Observations on the brooding behavior of *Sp. morchi* have led to the conclusion that spawning takes place when the animal is completely withdrawn into its tube.

4. An explanation as to how oocytes are deposited into the opercular brood pouch is advanced.

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